Phytochemical-rich antioxidant extracts of *Vaccinium vitis-idaea* L. leaves inhibit the formation of toxic Maillard reaction products in food models

Ieva Račkauskienė
Audrius Pukalskas
Alberto Fiore
Antonio Dario Troise
Petras Rimantas Venskutonis

Full Title

Phytochemical-rich antioxidant extracts of *Vaccinium vitis-idaea* L. leaves inhibit the formation of toxic Maillard reaction products in food models

Name(s) of Author(s)

Ieva Račkauskienė,[a] Audrius Pukalskas,[a] Alberto Fiore,[b] Antonio Dario Troise,[c] Petras Rimantas Venskutonis[*a]

Author Affiliation(s)

[a]Department of Food Science and Technology, Kaunas University of Technology, Radvilėnų pl. 19, LT-50254 Kaunas, Lithuania

[b]Division of Food and Drink, Engineering and Technology, Abertay University, Dundee DD1 1HG, UK

[c]Department of Agriculture, University of Naples "Federico II", Parco Gussone Ed. 84, 80055 Portici, Naples, Italy

Contact information for Corresponding Author

Address: Department of Food Science and Technology, Kaunas University of Technology, Radvilėnų pl. 19, LT-50254 Kaunas, Lithuania.

Phone: +37037456647.

Fax: +37037300155.

E-mail: rimas.venskutonis@ktu.lt

Word count of text

7220 words

Short version of title

Lingonberry extract in Maillard reaction

Choice of journal/section

Food Chemistry (also relevant to Toxicology and Chemical Food Safety)

Author disclosures

We declare the there are no conflicts of interests

ABSTRACT: Thermal treatment of proteinaceous foods generates heat-induced Maillard reaction substances including toxic advanced glycation end products (AGEs) and heterocyclic amines (HAs). It is known that plant phenolic compounds may influence Maillard reaction. This study investigated the impact of lingonberry leaf extracts on the formation of N^{ε}-(carboxymethyl)lysine (CML) and N^{ε}-(2-furoylmethyl)-L-lysine (furosine) in milk model system and HAs in meat-protein and meat model systems. In addition, lingonberry leaf extracts obtained by different solvents were characterized by radical scavenging, Folin-Ciocalteu assays and ultra-high pressure liquid chromatography quadruple-time-of flight mass spectrometry (UPLC-qTOF-MS). Water extract (WE) stronger suppressed CML than furosine formation in milk model system: CML levels were reduced by nearly 40%. Moreover, quinic acid and catechin which were abundant in WE, were effective in inhibiting CML and furosine formation. WE and acetone extract (AE) at 10 mg/mL significantly inhibited HAs formation in both model systems. However, higher suppressing effect on HAs formation showed AE which had lower antioxidant capacity and total phenolic content values than WE. WE contained higher amounts of hydroxycinnamic acids, proanthocyanidins and flavonols, while AE was richer in flavan-3-ols and arbutin derivatives. It indicates that the composition of phenolics might be a major factor for explaining different effect of extracts from the same plant on HAs formation. In general, the results suggest that lingonberry leaves is a promising source of phytochemicals for inhibiting toxic Maillard reaction products and enriching foods with plant bioactive compounds.

Keywords: *Vaccinium vitis-idaea* L. leaves; Maillard reaction; CML; furosine; heterocyclic amines

Practical Application:

The increased consumption in processed foods has been linked with the increased risks of various diseases, while thermal food processing is required to develop flavour, insure safety and extend shelf life. Therefore, developing effective technological means for inhibiting the formation of heat-induced toxic substances is an important task. This study showed a potential of lingonberry leaf extracts containing health beneficial phytochemicals to suppress the formation of toxic Maillard reaction products during heating of milk and meat.

Introduction

Thermal treatment of raw milk or meat during processing is an essential step in reducing microbiological contamination causing foodborne diseases and extending product shelf life. On the other hand, thermal treatment results in the formation of undesirable compounds demonstrating toxicity and allergenicity. The majority of those modifications are linked to the Maillard reaction occurring between carbonyl groups of reducing sugars and free amino groups of amino acids (Nursten, 2005).

Thermal treatment of raw milk in such processing steps as separation, normalization, homogenization, pasteurization, sterilization or ultra-heat treatment induces Maillard reaction mainly between lactose and lysine residues and generates the advanced glycation end products (AGEs). The formation of AGEs increases with increasing heating time and temperature. The presence of AGEs in foods contributes to the increased inflammation and oxidative stress (Uribarri et al., 2007), which are linked to the recent epidemics of diabetes and cardiovascular diseases (Birlouez-Aragon et al., 2010; Sandu et al., 2005). A range of dietary AGEs compounds have been reported, while the present study is focused on N^{ε}-(carboxymethyl)lysine (CML) and indirect marker, N^{ε}-(2-furoylmethyl)-L-lysine (furosine). CML is one of the most studied AGE, which is present in both biological systems, such as plasma, urine, tissues, skin collagen and in many heat-processed foods (Nguyen, van der Fels-Klerx, & van Boekel, 2014). It is generated from the oxidation of Amadori product (AP) or direct reaction of lysine ε-amino group with glyoxal produced by degradation/oxidation of sugars during thermal treatment and lipid peroxidation. Furosine is an indirect marker of AGEs indicating early stage of Maillard reaction

and is formed from the acid hydrolysis of the APs (e.g. fructosyl-lysine) (Henle, Zehetner, & Klostermeyer, 1995).

Thermal treatment of meat, particularly by deep-frying, oven-frying, roasting, grilling and searing generates the formation of heterocyclic amines (HAs) possessing mutagenic and carcinogenic properties. They are formed mainly as Maillard reaction products from amino acids, creatin(in)e and reducing sugars (Skog, Johansson, & Jägerstad, 1998). Depending on the cooking method, heating temperature and time, as well as on the content of precursors the formation of different HAs are favoured during thermal treatment (Gibis & Weiss, 2015; Linghu, Karim, & Smith, 2017; Yu et al., 2018). 2-Amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP) is the most abundant mutagenic HA of meat, while the chicken was reported as the most susceptible type of meat for the formation of this HA (Gibis & Weiss, 2015). It was observed that PhIP is carcinogenic in the rat colon, mammary gland, prostate, and breast (Shirai et al., 1997). It was also shown that many HAs possess carcinogenic/mutagenic/genotoxic effects both *in vitro* (Dumont et al., 2010) and *in vivo* (Chao et al., 2005; Shirai et al., 1997). Recent studies have supported positive associations of HA's with colorectal adenoma risk (Budhathoki et al., 2015; Khan et al., 2019) and increased oxidative stress in humans (Carvalho et al., 2015).

Phenolic compounds have been shown to possess inhibiting effects on the formation of AGEs and HAs (Puangsombat, Jirapakkul, & Smith, 2011). These effects have been explained by different reaction mechanisms, namely radical scavenging (Kikugawa, 1999), amine group blocking (Guerra & Yaylayan, 2014), scavenging dicarbonyl intermediates (Totlani & Peterson, 2005) or reactive carbonyls produced from amino acid degradation (Cheng et al., 2009) and lipid oxidation (Hidalgo, Delgado, & Zamora, 2017). For instance, catechin (Kokkinidou &

Peterson, 2014), (–)-epicatechin (Totlani & Peterson, 2006), (–)-epigallocatechin-3-gallate (EGCG) (Bin, Peterson, & Elias, 2012) inhibited AGEs by trapping dicarbonyl compounds. EGCG inhibited PhIP by scavenging reactive carbonyls from phenylacetaldehyde degradation (Cheng et al., 2009). Moreover, the structure of phenolics was explored as an important factor in the inhibition of HAs formation (Salazar, Arámbula-Villa, Hidalgo, & Zamora, 2014). Therefore, phenolic antioxidant-rich natural plant extracts may be useful additives in proteinaceous foods for inhibiting toxic Maillard reaction products.

Lingonberry (*Vaccinium vitis-idaea* L., Ericaceae) is one of the most important wild berry plant in the forests of the Nordic countries. Lingonberry leaves have been used in traditional medicine for their diuretic, astringent, and antiseptic properties to treat urinary tract infections. Lingonberry leaves contain many classes of flavonoids such as flavan-3-ols, flavanols, hydroxycinnamic acids, and proanthocyanidins (Hokkanen, Mattila, Jaakola, Pirttilä, & Tolonen, 2009; Ieri, Martini, Innocenti, & Mulinacci, 2013; Liu et al., 2014). According to European Commission Novel Food Catalogue lingonberry leaves have been authorized as food supplements. In addition, *V. vitis-idaea* berry extracts demonstrated antiglycation activity (Beaulieu et al., 2010), while there are no reports on antiglycation properties or HAs inhibition by lingonberry leaf extracts.

So far as milk and meat are very important foods for adequate human nutrition, particularly in supplying high value proteins and various bioactive micronutrients, the application of plant antioxidants for reducing the formation of undesirable toxic compounds during heat treatment might be a promising approach in achieving better equilibrium between the positive and negative nutritional aspects in the consumption of heat processed proteinaceous foods.

Therefore, this study was aimed at investigating the effect of lingonberry leaf extracts on the formation of CML and furosine in milk model system and HAs in meat-protein and meat model systems. The antioxidative potential of different lingonberry leaf extracts obtained by different solvents from deodorized and non-deodorized material was characterized by radical scavenging capacity, the total phenolic content and composition of phenolic compounds.

Materials and methods

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), 2,2-azinobis-3-ethyl benzothiazoline-6-sulphonic acid (ABTS), 6-hydroxy-2,5,7,8,-tetramethylchromane-2-carboxylic acid (Trolox), 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH), diethylene glycol, sodium borohydride, boric acid, nonafluoropentanoic acid (NFPA), trichloroacetic acid (TCA), D (+)-lactose monohydrate, acetonitrile (HPLC grade), rutin, quercetin-3-*O*-glucoside, quinic acid, *p*-coumaric, caffeic and gallic acids, Folin–Ciocalteu phenol reagent and Milli-Q water were from Sigma-Aldrich (St. Louis, MO, USA). (+)-Catechin reference was purchased from Chromadex (Irvine, California, USA), chlorogenic acid, L-phenylalanine and glucose were from Roth (Karlsruhe, Germany); fluorescein sodium salt (FL) from Fluka Chemicals (Steinheim, Germany). Heterocyclic amines, MeIQ (2-amino-3,4-dimethylimidazo[4,5-f]quinolone), MeIQx (2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline), Trp-P-1 (3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole) and PhIP were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). CML, d_2-CML and furosine were purchased from PolyPeptide Laboratories (Strasbourg, France). Creatinine was from Alfa Aesar (Karlsruhe, Germany). Bond Elut PRS LRC cartridges and Bond-

Elut C18 cartridges were purchased from Agilent Technologies (Lake Forest, CA, USA). Chromabond XTR cartridges were from Macherey-Nagel GmbH & Co. (Bethlehem, PA, USA). Oasis HLB cartridges for CML and furosine stable isotope dilution assay were purchased from Waters Corporation (Milford, CA, USA).

Plant material and preparation of extracts

Vaccinium vitis-idaea L. leaves were collected in the forest of Mažeikiai (Telšiai district, Lithuania) from June to mid-September. The plants were dried at 40 °C and ground in an ultra-centrifugal rotor mill Retsch ZM200 (Retsch GmbH, Haan, Germany) using 0.5 mm sieve. Powdered lingonberry leaves (200 g) were placed in a 3 L round-bottom flask, connected with Clevenger-type apparatus, diluted with 1.5 L of water and hydrodistilled during 3 h for removing volatile compounds. Then, water phase was separated from the solids by filtering through the cotton and freeze-dried. The product obtained is indicated as water extract (WE). The solid residues, which are called deodorized lingonberry leaves, were dried at 40 °C in a drying oven. Then, raw and deodorized lingonberry leaves were subjected to pressurised liquid extraction with methanol and acetone in a Dionex ASE 350 apparatus (Sunnyvale, CA, USA) at the following parameters: 70 °C, 10 MPa, 3 cycles, 5 min each, 100% flush volume, purging with nitrogen. Organic solvents were evaporated at 40 °C in a rotary vacuum evaporator (Büchi, Flawil, Switzerland). Acetone, as an aprotic lower polarity solvent, and methanol, as a protic high polarity solvent, were selected in order to evaluate what kind of compounds may be recovered by using these solvents and what extracts (containing lower or higher polarity phytochemicals) possess the strongest radical scavenging properties. In total, five extracts were

produced: three extracts from the raw (WE, ME, AE) and two extracts from the deodorized lingonberry leaves (DME and DAE).

Evaluation of antioxidant potential of lingonberry leaves

Folin-Ciocalteu assay

Total phenolic content was determined using Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999). Extract solution (0.2 mL) was added to 1.0 mL of Folin–Ciocalteu reagent (1:10, v/v) and was shaken for 5 min; then 0.8 mL of 7.5 mg/g sodium carbonate solution was added and after incubation in the dark for 1.5 h, the absorbance was read at 760 nm in a HALO RB-100 UV–Vis spectrophotometer (Dynamica, Switzerland). The results were expressed as mg gallic acid equivalents (GAE) in g of extract dry weight (edw) and in plant dry weight (pdw).

DPPH$^{\bullet}$ scavenging assay

DPPH$^{\bullet}$ scavenging was determined according to Brand-Williams, Cuvelier, & Berset (1995). Briefly, the decrease in absorbance was recorded at 517 nm in a FLUOstar Omega microplate reader (BMG Labtech, Durham, NC) over 35 min after the addition of 7.5 µL of extract or trolox solution in 96-well microplates with 300 µL of methanolic DPPH$^{\bullet}$ solution (60 µM). The results were expressed as effective concentrations EC_{50} (mg/mL) required to decrease the initial DPPH$^{\bullet}$ concentration in the reaction mixture by 50%.

ABTS$^{\bullet+}$ scavenging assay

196 The $ABTS^{\bullet+}$ scavenging (decolouration) capacity was determined according to Re et al.
197 (1999). Briefly, after adjusting the absorbance of $ABTS^{\bullet+}$ solution to 0.70±0.02 at 734 nm with
198 phosphate buffer (pH 7.4), the measurements were performed in microplate reader after
199 mixing 3 µL of extract or trolox solutions with 297 µL of diluted $ABTS^{\bullet+}$ solution. Trolox
200 equivalent antioxidant capacity (TEAC) was used to express $ABTS^{\bullet+}$ scavenging capacity in mM
201 TE/g edw and pdw.
202
203 **Oxygen radical absorbance capacity (ORAC) assay**
204 The ORAC assay was performed as described by Huang, Ou, Hampsch-Woodill, Flanagan, &
205 Prior (2002). Briefly, 25 µL of extract or trolox solution were mixed with 150 µL of 96 mM FL in
206 microplate, which further was incubated at 37 °C for 15 min. Then, 26 µL of 240 mM AAPH was
207 added and microplate was shaken for 30 s. The fluorescence was recorded (λ_{ex} = 493 nm, λ_{em} =
208 515 nm) every 66 s during 80 min. The ORAC values are expressed as mM TE/g edw and pdw.
209
210 **Analysis of phenolic compounds**
211 Phenolic compounds were analyzed by ultra-high pressure liquid chromatography with
212 quadruple-time of flight mass spectrometry (UPLC-qTOF-MS) using an Acquity UPLC system
213 (Waters Corporation) and Brüker maXis UHR-TOF mass spectrometer (Brüker Daltonics,
214 Bremen, Germany). Separation was performed using 1.7 µm Acquity BEH-C18 (50 × 2.1 mm)
215 column at a flow rate of 0.4 mL/min (injection volume 2 µL). The eluents were 0.1% formic acid
216 (A) and acetonitrile (B) at the following gradient: 0–2 min, 10–22% B, 2–7 min, 22%–50% B, 7–
217 10 min, 10% B. Mass spectra were acquired in a negative electrospray ionization mode (ESI) by

full scan acquisition covering a range of m/z 100-800 at the following parameters: capillary voltage, 4000 V; collision cell energy, 8 eV, dry gas temperature, 200 °C with a flow rate of 10 L/min; nebulizer pressure, 2 bar; drying and nebulizing gas, nitrogen. Peak identification was performed by comparing retention times (t_R) with those of standards or and accurate m/z values from which the molecular ion formulas were calculated for each compound. Mass fragmentation was performed with nitrogen as a collision gas at 40–45 eV collision energy.

Determination of effects of lingonberry leaf **extracts on** Maillard reaction products

Preparation of milk model system and determination of furosine and CML

Milk model system was prepared by dissolving (w/w) 1.2% skimmed milk powder, 2.5% whey protein (both from Lactalis Ingredients, Bourgbarré, France) and 5% lactose monohydrate in 87.9% of water, and then homogenizing (60 °C, 16 MPa, 3 passes) with 3.3% of palm oil (Kerfoot Groop, Yorkshire, UK) and 0.1% of soy lecithin Solec (DuPont, Dangé Saint Romain, France). WE, quinic acid and catechin were added at 0.05, 0.1 and 0.3 mg/mL concentrations, while the controls (also in other model systems) were without any additives. The samples were heated at 140 °C for 0, 30 and 45 s. Furosine and CML were extracted and determined as described by Delatour et al. (2009) and Fenaille et al. (2006) with slight modifications reported by Račkauskienė et al. (2015).

Preparation of meat-protein model system and determination of HAs

Meat-protein model system consisting of 0.4 mM phenylalanine, 0.4 mM creatinine and 0.2 mM glucose in 3 mL of diethylene glycol (14% of water) was used as described by Wong, Cheng,

& Wang (2012). WE and AE powders were added to the mixture at 0.1, 1, 5, and 10 mg/mL levels. The samples were heated at 128 °C in the closed stainless steel vials (homemade from stainless steel type EN 1.4401) for 120 min, afterwards cooled in an ice-water bath for 10 min and stored at −18 °C. After extraction of HAs (Wong, Cheng, & Wang, 2012) the eluate was dried under nitrogen and re-dissolved in 400 µL of methanol before analysis.

PhIP was determined using the same UPLC system as for phenolic compounds. Separation of HAs was performed using Acquity BEC-C18 column thermostated at 40 °C. The mobile phase was composed of 30 mM formic acid/ammonium formate (pH 4.75) in deionized water (A) and acetonitrile (B) delivered at 0.4 mL/min (injection volume 5 µL) at the following gradient: 0–0.15 min, 5% B; 0.15–2.5 min, 5–30% B; 2.5–3.0 min, 30–60% B; 3–5 min, 60% B and 5–7 min 60–100% B. Positive ESI was used for mass analysis and MS parameters were the same as described above. The full scan MS data were recorded in the range of 100 to 800 m/z. PhIP was quantified using calibration curve prepared from its solutions in methanol (1 - 800 ng/mL) and following the whole procedure described above. The limits of detection (LOD) and quantification (LOQ) were 1 and 3 ng/mL, respectively, while average PhIP recovery was approx. 61%.

Preparation of meat model system and determination of HAs

A meat model system consisting of 0.1 g of homogenized freeze-dried beef meat purchased in the local market (Kaunas, Lithuania) and 1 mL of diethylene glycol (14% of water) was used (Messner & Murkovic, 2004). WE and AE powders were added to the mixture at 0.1, 1, 5, and 10 mg/mL concentrations. The samples were placed in the stainless steel vessels, which were

closed and heated at 180 °C for 30 min. After heating the vessels were cooled on ice (10 min) to terminate the reaction and the samples were stored at −18 °C prior to extraction. The samples were then transferred to 50 mL glass beakers with 15 mL of 1 M NaOH and homogenized by magnetic stirring for 60 min at 500 rpm. Next, 5 mL of alkaline solution were mixed with diatomaceous earth in Chromabond XTR cartridges, and extracted (Wong et al., 2012). The eluate was dried under nitrogen and re-dissolved in 100 µL of methanol before analysis.

HAs were analysed on a Waters Acquity UPLC H-Class system equipped with triple quadruple spectrometer Waters Xevo TQ-S in a positive ESI mode. The compounds were separated in an Acquity BEH-C18 column at a flow rate of 0.8 mL/min (injection volume 2 µL). The mobile phase was 30 mM formic acid-ammonium formate buffer (A) and acetonitrile (B) eluted at the following gradient: 0–0.1 min, 5% B; 0.1–1.5 min, 5–30% B; 1.5–1.8 min, 30–60% B; 1.8–1.85 min, 60% B; 1.85–2.4 min, 60–95% B; 2.4–2.9 min, 5% B. The quantitation was carried out in multiple reaction monitoring (MRM) mode using transitions as follows: 225 → 210 for PhIP; 213 → 198 for MeIQ; 214 → 199 for MeIQx; 212 → 195 for TrP-P-1. MRM conditions were automatically optimized with 100 ng/mL HAs using the Intellistart function system. MS/MS parameters were as follows: cone voltage, 50 V; capillary voltage, 3.0 kV; desolvation temperature, 350 °C; cone gas flow rate, 150 L/h (Nitrogen); desolvation gas flow rate, 650 L/h (Nitrogen); collision gas flow rate, 0.13 mL/min (Argon); collision energy, 35 eV. Data acquisition was carried out by MassLynx 4.1 software. Quantitative analysis was performed using Waters TargetLynxTM software. Calibration curves ranged from 1 to 500 ng/mL. The LOD and LOQ for standard solutions were calculated based on signal-to-noise ratios of 3:1 and 10:1,

283 respectively. The LOD and LOQ values (ng/mL) were 1 and 2 for PhIP, 2 and 5 for MeIQ, 2 and 5
284 for MeIQx, and 2 and 6 for Trp-P-1, respectively. The recovery ranged from 67 to 86%.
285
286 **Statistical analysis**
287 Antioxidant characteristics are reported as means ± standard deviations (SD) from 3
288 replicate measurements. Milk, meat-protein and meat model systems were prepared in
289 duplicate, while extraction procedure was performed in duplicate for each replicate. Each
290 replicate was analysed in triplicate in LC-MS/MS and UPLC systems. Multivariance analysis with
291 Turkey test as a post hoc analysis was used for statistical data assessment ($P < 0.05$).
292
293 **Results and discussion**
294 **Evaluation of antioxidant properties of** lingonberry leaves
295 **Extraction yields and** total phenolic content **of** lingonberry leaf **extracts**
296 In case of raw lingonberry leaves the highest and the lowest extraction yields were obtained
297 with methanol and acetone, respectively, whereas deodorized lingonberry leaves gave 2-fold
298 lower extract yields than raw lingonberry leaves (Table 1). In general, the yields decreased in
299 the following order: ME > WE > AE > DME > DAE. The differences in the ME and WE yields were
300 not large; both solvents are high polarity compounds, whereas methanol is known as one of the
301 most effective solvents for the extraction of low sugar content plant materials. Moreover,
302 methanol extraction was performed at the increased pressure, which facilitates diffusion and
303 solubilization processes. Acetone is less effective solvent for higher polarity constituents, while
304 DME and DAE were prepared from the solid hydrodistillation residue, which has already been

extracted by water. The highest amounts of total phenolics expressed in mg GAE/g pdw were recovered with ME followed by WE, AE, DME, and DAE. More than 2-fold lower total phenolic content was determined for the deodorized than for the raw lingonberry leaf extracts. Recent study (Bujor, Ginies, Popa, & Dufour, 2018) reported total phenolic content for lingonberry leaves in the range of 85.3 - 114.6 mg GAE/g DM, which is in agreement with our data (57.42±1.19–91.15±1.3 mg GAE/g pdw).

Radical scavenging properties of lingonberry leaf **extracts**

DPPH$^{\bullet}$, ABTS$^{\bullet+}$ scavenging and ORAC values are presented in Table 1. DAE was the weakest DPPH$^{\bullet}$ scavenger while the differences between EC_{50} values of WE, ME, AE, and DME were not significant in this assay. It was also observed that lingonberry leaf extracts scavenged DPPH$^{\bullet}$ rather slowly; the reaction between antioxidants and radical was completed in approx. 35 min. In ABTS$^{\bullet+}$ and ORAC assays the antioxidant capacity of extracts was decreasing in the following order: WE > ME > AE > DME > DAE. A significant correlation exists between total phenolic content (mg GAE/g pdw) and DPPH$^{\bullet}$ scavenging (r = -0.70), ABTS$^{\bullet+}$ scavenging (r = 0.87) and ORAC (r = 0.95) values.

In general, high polarity protic solvents methanol and water gave higher yields, total phenolic content and antioxidant capacity values than lower polarity solvent acetone. It indicates that lingonberry leaves contain higher amounts of polar polyphenolic compounds. Therefore, extraction yields, total phenolic content and antioxidant capacity values of extracts obtained from the deodorized material were remarkably lower in comparison to the extracts from the raw material; the main part of water-soluble compounds remains in WE after hydro-

distillation of lingonberry leaves. It should be noted that comprehensive *in vitro* evaluation of radical scavenging capacity of different lingonberry leaf extracts is reported for the first time.

Comparison of phenolic profiles of different lingonberry leaf **extracts**

The UV chromatograms and the relative amounts (based on UPLC-qTOF-MS peak areas) of phenolic compounds in different lingonberry leaf extracts are presented in Figure 1 and Table 2. Twenty phenolic compounds were positively or tentatively identified in lingonberry leaf extract. Authentic standards were used for identification of catechin, quinic, chlorogenic, caffeic, citric and *p*-coumaric acids, rutin and quercetin-3-O-glucoside. Other compounds were tentatively identified by comparing the exact masses, fragmentation patterns, t_R by using databases such as METLIN and Human Metabolome (HMDB) (Benton, Wong, Trauger, & Siuzdak, 2008) and previously reported data for those compounds in lingonberry leaves (Ek, Kartimo, Mattila, & Tolonen, 2006; Liu et al., 2014). It should be noted that so far as standards were not available for these compounds their identification is assumed as tentative. ME and AE demonstrated lower recovery of phenolics, expressed in AU×10^{-7}/mg edw, than WE by 37 and 51%, respectively. Lower recovery of phenolics by acetone may be explained by the prevalence of higher polarity compounds in lingonberry leaves, which are better soluble in protic solvents. Finally, DME and DAE contained lower amounts of total phenols by 53 and 81% than WE, respectively. The content of phenolics in deodorized plant extracts was considerably lower comparing with the extracts from the raw lingonberry leaves, because the main part of polar compounds was dissolved in water during hydro-distillation. It indicates that after water

extraction of raw lingonberry leaves some part of extractable phenolics still remained in deodorized lingonberry leaves and were recovered by methanol and acetone.

The main classes of phenolics in WE were hydroxycinnamic acids and flavonols while arbutin derivatives and flavan-3-ols were more abundant in ME and AE. The dominant flavonols in all extracts were pentosides and hexosides of quercetin, which is in agreement with previous studies (Ek et al., 2006, Liu et al., 2014). Exact mass and fragmentation patterns indicated that the compounds ***16*** and ***17*** may be quercetin-3-*O*-xyloside and quercetin-3-*O*-arabinoside. This assumption is also supported by the order of their elution, which was determined in previous study (Liu et al., 2014). Caffeic acid ***7***, *p*-coumaric acid ***8*** and quercetin-pentoside ***16*** were found only in WE, DME and DAE indicating that detectable amounts of these compounds were released after hydro-distillation. Also, citric acid ***4***, chlorogenic acid ***6***, *B*-type proanthocyanidin ***12*** and rutin ***14*** were found only in WE.

The differences in the yields, antioxidant capacity and total phenols for WE and ME were not considerable. This is a positive result indicating that green and cheap solvent water may be used for preparing phenolics-rich extracts from lingonberry leaves on industrial scale. However, the profile of phenols in WE was different compared to ME and AE, while in the latter extracts it was quite similar. WE and AE were chosen for further evaluation of their possible inhibition of toxic Maillard reaction products.

Effects of lingonberry leaves **on** Maillard reaction products

The effect of WE, quinic acid and catechin on furosine and CML formation

In this experiment, the antiglycation activity of WE, quinic acid and catechin was accessed through the furosine and CML determination in milk model system. Since WE exhibited the highest antioxidant potential it was chosen for this experiment. To identify possible active components in the lingonberry leaves, the standards of quinic acid and catechin, were further evaluated. Quinic acid was among the major constituents in WE, while catechin was the strongest radical scavenger as it was determined by the on-line HPLC-DPPH$^{\bullet}$ scavenging method (data not shown).

The effect of WE on furosine and CML formation is represented in Figure 2A1 and A2, respectively; the former was better inhibited at lower, while the latter one at higher concentrations of WE. For instance, at 0.05 and 0.1 mg/mL WE reduced furosine level by 32 and 21% after 30 s of heating and by 18 and 27% after 45 s of heating, respectively. CML formation was reduced by 38 and 19% after 30 s of heating and by 42 and 38% after 45 s of heating at 0.1 and 0.3 mg/mL WE addition, respectively.

The effect of quinic acid and catechin on furosine and CML formation is presented in Figure 2B and C, respectively. Quinic acid and catechin reduced the levels of furosine in milk model system after 30 s heating by 35% at all concentrations; while after 45 s heating, its level was reduced by approx. 10 and 40% at 0.05 mg/mL and 0.3 mg/mL concentrations, respectively. The formation of CML was even more efficiently inhibited than furosine. The average decrease of CML at both heating times was approx. by 31, 29, and 43%, and 31, 39, and 51% when 0.05, 0.1 and 0.3 mg/mL of quinic acid (Figure 2B2) and catechin (Figure 2C2) were added, respectively. The reduction of CML and furosine by catechin can be explained through two pathways: first, through the reaction between amino groups and *o*-quinoidal moieties firming via B ring

391 oxidation; and second, through the trapping of carbonyl-containing sugar fragments at highly
392 activated A ring. There are no reports showing suppressing effect of quinic acid on Maillard
393 reaction products formation in milk. However, the protecting effect against protein
394 carbonylation was reported previously (Yoshimura et al., 2016). Quinic acid is a cyclic hydroxyl-
395 acid that is present in various fruits and vegetables; therefore, it would be interesting to
396 continue the studies for clarifying reducing effect of quinic acid on the formation of Maillard
397 reaction products.

398 In general, the inhibition effect of WE on Maillard reaction products formation depended on
399 heating time and extract concentration. The reduction of both Maillard reaction products can
400 be associated with the above-described ways. Given their antiglycation activity and relatively
401 high abundance in leaves, catechin and quinic acid may be suggested as the main active
402 compounds responsible for CML and furosine inhibition. Catechin, quercetin-3-*O*-galactoside
403 and cyanidin-3-*O*-glucoside were reported as the main contributors to the antiglycation
404 properties of *V. vitis-idaea* berry extracts (Beaulieu et al., 2010).

405 **The effect of WE and AE on the formation of HAs**

406 In this experiment, the effects of WE and AE of lingonberry leaves on the formation on HAs
407 were tested in two model systems: meat-protein and meat. In a meat-protein model system,
408 the interference of meat matrix components (lipids, proteins, water) was eliminated, by using
409 only the substances, which are necessary for the formation of HAs. As only phenylalanine was
410 used, mainly PhIP formation was generated; therefore the effect of lingonberry leaf extracts on
411 the formation of PhIP was evaluated in meat-protein model system. Meat model system

412 consisted of a real meat-like matrix, from which only the water factor was eliminated; in this
413 case the formation of HAs was evaluated measuring several typical to roasted beef HAs.
414 The effect of lingonberry leaves extracts on PhIP formation in meat-protein model system is
415 shown in Figure 3. The amounts of PhIP significantly increased when WE were added in the
416 range of 0.1-5 mg/mL and decreased by 20% with the addition of 10 mg/mL; while significant
417 effect of AE was observed only at 10 mg/mL when PhIP level was reduced by 40%. Better
418 inhibitory effect observed in case of AE, possessing weaker radical scavenging and total
419 phenolic content than WE, may seem somewhat unexpected. According to our results and
420 previously reported observations (Hidalgo & Zamora, 2018), it may be assumed that different
421 effect of WE and AE on PhIP formation could be due to the different composition of phenolics:
422 WE contained more hydroxycinnamic acids, proanthocyanidins and flavonols than AE. The
423 amounts of hydroxycinnamic acids as quinic, protocatechuic, chlorogenic, caffeic and *p*-
424 coumaric were in traces or even undetectable in AE. Phenolic acids, e.g. chlorogenic (Cheng,
425 Chen, & Wang, 2007), were shown to be ineffective inhibitors or even enhancers of PhIP
426 formation. Moreover, AE comparing to WE contained higher amounts of flavan-3-ols such as
427 catechin and chinchonain *1* and arbutin derivative (2-O-caffeoylarbutin). Green tea catechins in
428 many studies were reported as effective inhibitors of HAs (Cheng et al., 2009; Quelhas et al.,
429 2010). Those differences in the composition of phenolics explain different effect of lingonberry
430 leaf extracts on PhIP formation in the analysed meat-protein model system.
431 The effects of lingonberry leaves extracts on the formation of four HAs in meat model
432 system are presented in Figure 4. In this model four HAs were quantified; the dominant
433 compound was PhIP (266.84±6.67 ng/g of beef dry weight), whereas MeIQ, MeIQx and Trp-P-1

were found in remarkably lower amounts. Trp-P-1 is a carboline type HA and in cooked meat usually forms at higher temperatures (> 200 °C); however, probably due to prolonged heating (120 min) it was also formed in the used beef-model system. WE and AE reduced PhIP concentration dose-dependent by 3.24, 10.55, 39.29 and 14.42, 26.85, 37.43%, respectively. Consequently, the opposite effects of WE addition on the formation of PhIP were observed in the more real-like meat model system: WE did not promote PhIP formation. It may be explained by the different formation mechanisms of PhIP in chemical and real-like meat model systems, as was reported previously (Cheng et al., 2007). However, the levels of MeIQ, MeIQx and Trp-1 were increased in the samples with lingonberry leaf extracts and WE showed stronger promoting effect than AE. The addition of 10 mg/mL of WE showed the highest promoting effect on the formation of MeIQ, MeIQx and Trp-1 and the highest reducing effect on PhIP formation. These results might be explained by different HAs formation mechanisms, which largely depend on their structures. For instance, MeIQ belongs to imidazoquinoline, while MeIQx to imidazoquinoxaline class of Has. The precursors of MeIQ and MeIQx are pyridine and pyrazine free radicals, respectively, forming *via* Maillard reaction between sugars and amino acids during Strecker degradation. Catechin and EGCG influenced the formation of pyrazinium radicals by reacting with imine intermediates: moreover, at low concentrations (5-20 mM) it increased and at high concentrations (≥50 mM) decreased their formation. Thus, the formation of MeIQ and MeIQx could be enhanced through the generation of pyridine and pyrazine radicals. As PhIP belongs to imidazopyridine type compound, it is formed from the condensation of phenylacetaldehyde and creatinine. EGCG has been shown as an effective

455 inhibitor of phenylacetaldehyde, which is an important intermediate product in PhIP formation
456 (Cheng et al., 2009).
457 Based on the obtained data, we can assume that WE may act as a generator of Trp-P-1,
458 MeIQ and MeIQx precursors, and as a scavenger of intermediates in the formation of PhIP in
459 meat model system. In general, AE was more potential additive for reducing HAs formation in
460 both model systems.
461
462 **Conclusions**
463 The results of our study indicate that lingonberry leaf extracts possess the ability of tuning
464 Maillard reaction in food models. WE inhibited CML and furosine formation in milk model
465 system during its heating; moreover the inhibitory effect was stronger for the formation of CML
466 than furosine. This finding suggests that WE is stronger inhibitor of the advanced stage Maillard
467 reaction products compared to the early stage products. Phenolic compounds such as catechin
468 and quinic acid, which were abundant in WE, showed strong suppressing effect on CML and
469 furosine formation in milk model system; consequently, these compounds may be important
470 lingonberry leaves extract constituents responsible for CML and furosine inhibition. Results of
471 extract's effects in meat models systems suggest that inhibition effect is more related to the
472 phenolic compounds profile than to the radical scavenging capacity or total phenolic content.
473 Accordingly, it may be preliminary hypothesized that hydroxycinnamic acids might act as
474 enhancers, while flavan-3-ols and arbutin derivatives as inhibitors of HAs. Moreover, effective
475 phytochemical composition of such extracts could be purposively designed by using different
476 organic solvents and procedures for extractions. In general, the results obtained show that

lingonberry leaf extracts are promising ingredients for their applications in foods both for controlling the formation of Maillard reaction products during thermal processing and for enriching them with natural antioxidants possessing health benefits.

In addition, the results supports the need of further studies for the evaluation of the mechanisms of the involvement of plant phytochemicals in the Maillard reaction to ensure optimal application of natural plant origin ingredients in suppressing the formation of heat-induced toxic compounds in foods.

Acknowledgments

The study was supported by Research Council of Lithuania, grant no. S-MIP-17-100.

Author Contributions

I. Račkauskienė performed the major part of experimental work (data acquisition, statistical analysis and data interpretation) and prepared the manuscript draft and finalized it after approval by the other co-authors. P.R. Venskutonis conceptualized and designed the study, critically reviewed the data and finally approved the manuscript. A. Pukalskas, A. Fiore and A.D. designed the study, performed part of experimental work, interpreted data, critically reviewed it and finally approved the manuscript.

References

Beaulieu, L., Harris, C. S., Saleem, A., Cuerrier, A., Haddad, P. S., Martineau, L. C., . . . Arnason, J. T. (2010). Inhibitory effect of the Cree traditional medicine Wiishichimanaanh (*Vaccinium vitis-idaea*) on advanced glycation endproduct formation: Identification of active principles. *Phytotherapy Research,* 24(5), 741-747. https://doi.org/10.1002/ptr.3025

Benton, H. P., Wong, D. M., Trauger, S. A., Siuzdak, G. (2008). XCMS2: Processing tandem mass spectrometry data for metabolite identification and structural characterization. *Analytical Chemistry*, 80(16), 6382-6389.

Bin, Q., Peterson, D. G., & Elias, R. J. (2012). Influence of phenolic compounds on the mechanisms of pyrazinium radical generation in the Maillard reaction. *Journal of Agricultural and Food Chemistry,* 60(21), 5482-5490. https://doi.org/10.1021/jf3010838

Birlouez-Aragon, I., Saavedra, G., Tessier, F. J., Galinier, A., Ait-Ameur, L., Lacoste, F., Niamba, C. N., Alt, N., Somoza, V., & Lecerf, J. M. (2010) A diet based on high-heat-treated foods promotes risk factors for diabetes mellitus and cardiovascular diseases. *The American Journal of Clinical Nutrition,* 91(5), 1220-1226. https://doi.org/10.3945/ajcn.2009.28737

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology,* 28(1), 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

Budhathoki, S., Iwasaki, M., Yamaji, T., Sasazuki, S., Takachi, R., Sakamoto, H., . . . Tsugane, S. (2015). Dietary heterocyclic amine intake, NAT2 genetic polymorphism, and colorectal adenoma risk: The colorectal adenoma study in Tokyo. *Cancer Epidemiology, Biomarkers & Prevention,* 24(3), 613-620. https://doi.org/10.1158/1055-9965.EPI-14-1051

Bujor, O., Ginies, C., Popa, V. I., & Dufour, C. (2018). Phenolic compounds and antioxidant activity of lingonberry (*Vaccinium vitis-idaea* L.) leaf, stem and fruit at different harvest periods. *Food Chemistry,* 252, 356-365. https://doi.org/10.1016/j.foodchem.2018.01.052

Carvalho, A. M., Miranda, A. M., Santos, F. A., Loureiro, A. P. M., Fisberg, R. M., & Marchioni, D. M. (2015). High intake of heterocyclic amines from meat is associated with oxidative stress. *British Journal of Nutrition,* 113(8), 1301-1307. https://doi.org/10.1017/S0007114515000628

Chao, A., Thun, M. J., Connell, C. J., McCullough, M. L., Jacobs, E. J., Flanders, W. D., . . . Calle, E. E. (2005). Meat consumption and risk of colorectal cancer. *JAMA,* 293(2), 172-182. https://doi.org/10.1001/jama.293.2.172

Cheng, K. W., Chen, F., & Wang, M. (2007). Inhibitory activities of dietary phenolic compounds on heterocyclic amine formation in both chemical model system and beef patties. *Molecular Nutrition & Food Research,* 51(8), 969-976. https://doi.org/10.1002/mnfr.200700032

Cheng, K. W., Wong, C. C., Chao, J., Lo, C., Chen, F., Chu, I. K., . . . Wang, M. (2009). Inhibition of mutagenic PhIP formation by epigallocatechin gallate via scavenging of phenylacetaldehyde. *Molecular Nutrition & Food Research,* 53(6), 716-725. https://doi.org/10.1002/mnfr.200800206

534 Delatour, T., Hegele, J., Parisod, V., Richoz, J., Maurer, S., Steven, M., & Buetler, T. (2009). Analysis
535 of advanced glycation endproducts in dairy products by isotope dilution liquid
536 chromatography–electrospray tandem mass spectrometry. the particular case of
537 carboxymethyllysine. *Journal of Chromatography A,* 1216(12), 2371-2381.
538 https://doi.org/10.1016/j.chroma.2009.01.011

539 Dumont, J., Jossé, R., Lambert, C., Anthérieu, S., Le Hegarat, L., Aninat, C., . . . Guillouzo, A. (2010).
540 Differential toxicity of heterocyclic aromatic amines and their mixture in metabolically
541 competent HepaRG cells. *Toxicology and Applied Pharmacology,* 245(2), 256-263.
542 https://doi.org/10.1016/j.taap.2010.03.008

543 Ek, S., Kartimo, H., Mattila, S., & Tolonen, A. (2006). Characterization of phenolic compounds
544 from lingonberry (*Vaccinium vitis-idaea*). *Journal of Agricultural and Food Chemistry,* 54(26),
545 9834-9842. https://doi.org/10.1021/jf0623687

546 Fenaille, F., Parisod, V., Visani, P., Populaire, S., Tabet, J., & Guy, P. A. (2006). Modifications of
547 milk constituents during processing: A preliminary benchmarking study. *International Dairy*
548 *Journal,* 16(7), 728-739. https://doi.org/10.1016/j.idairyj.2005.08.003

549 Gibis, M., & Weiss, J. (2015). Impact of precursors creatine, creatinine, and glucose on the
550 formation of heterocyclic aromatic amines in grilled patties of various animal species. *Journal*
551 *of Food Science,* 80(11), C2430-C2439. https://doi.org/10.1111/1750-3841.13090

552 Guerra, P. V., & Yaylayan, V. A. (2014). Interaction of flavanols with amino acids: Postoxidative
553 reactivity of the B-ring of catechin with glycine. *Journal of Agricultural and Food Chemistry,*
554 62(17), 3831-3836. https://doi.org/10.1021/jf5005989

555 Henle, T., Zehetner, G., & Klostermeyer, H. (1995). Fast and sensitive determination of furosine.
556 *Zeitschrift Für Lebensmittel-Untersuchung Und Forschung,* 200(3), 235-237.
557 https://doi.org/10.1007/BF01190503

558 Hidalgo, F. J., Delgado, R. M., & Zamora, R. (2017). Protective effect of phenolic compounds on
559 carbonyl-amine reactions produced by lipid-derived reactive carbonyls. *Food Chemistry,* 229,
560 388-395. https://doi.org/10.1016/j.foodchem.2017.02.097

561 Hidalgo, F. J., & Zamora, R. (2018). 2,4-alkadienal trapping by phenolics. *Food Chemistry,* 263, 89-
562 95. https://doi.org/10.1016/j.foodchem.2018.04.121

563 Hokkanen, J., Mattila, S., Jaakola, L., Pirttilä, A. M., & Tolonen, A. (2009). Identification of phenolic
564 compounds from lingonberry (*Vaccinium vitis-idaea* L.), bilberry (*Vaccinium myrtillus* L.) and
565 hybrid bilberry (*Vaccinium x Intermedium ruthe* L.) leaves. *Journal of Agricultural and Food*
566 *Chemistry,* 57(20), 9437-9447. https://doi.org/10.1021/jf9022542

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry,* 50(16), 4437-4444. https://doi.org/10.1021/jf0201529

Ieri, F., Martini, S., Innocenti, M., & Mulinacci, N. (2013). Phenolic distribution in liquid preparations of *Vaccinium myrtillus* L. and *Vaccinium vitis idaea* L. *Phytochemical Analysis,* 24(5), 467-475. https://doi.org/10.1002/pca.2462

Khan, I. A., Yiqun, C., Zongshuai, Z., Ijaz, M. U., Brohi, S. A., Ahmad, M. I., . . . Huang, M. (2019). Occurrence of heterocyclic amines in commercial fast-food meat products available on the chinese market and assessment of human exposure to these compounds. *Journal of Food Science,* 84(1), 192-200. https://doi.org/10.1111/1750-3841.14418

Kikugawa, K. (1999). Involvement of free radicals in the formation of heterocyclic amines and prevention by antioxidants. *Cancer Letters,* 143(2), 123-126. https://doi.org/10.1016/S0304-3835(99)00140-8

Kokkinidou, S., & Peterson, D. G. (2014). Control of maillard-type off-flavor development in ultrahigh-temperature-processed bovine milk by phenolic chemistry. *Journal of Agricultural and Food Chemistry,* 62(32), 8023-8033. https://doi.org/10.1021/jf501919y

Linghu, Z., Karim, F., & Smith, J. S. (2017). Amino acids inhibitory effects and mechanism on 2-amino-1-methyl-6-phenylimidazo 4,5-b]pyridine (PhIP) formation in the Maillard reaction model systems. *Journal of Food Science,* 82(12), 3037-3045. https://doi.org/10.1111/1750-3841.13959

Liu, P., Lindstedt, A., Markkinen, N., Sinkkonen, J., Suomela, J., & Yang, B. (2014). Characterization of metabolite profiles of leaves of bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.). *Journal of Agricultural and Food Chemistry,* 62(49), 12015-12026. https://doi.org/10.1021/jf503521m

Messner, C., & Murkovic, M. (2004). Evaluation of a new model system for studying the formation of heterocyclic amines. *Journal of Chromatography B,* 802(1), 19-26. https://doi.org/10.1016/j.jchromb.2003.11.015

Nguyen, H. T., van der Fels-Klerx, H. J., & van Boekel, M. A. J. S. (2014). N^{ε}-(carboxymethyl)lysine: A review on analytical methods, formation, and occurrence in processed food, and health impact. *Food Reviews International,* 30(1), 36-52. https://doi.org/10.1080/87559129.2013.853774

Nursten, H. E. (2005). *The Maillard Reaction: Chemistry, Biochemistry and Implications* (pp. 5–100). Royal Society of Chemistry, Cambridge

601 Puangsombat, K., Jirapakkul, W., & Smith, J. S. (2011). Inhibitory activity of Asian spices on
602 heterocyclic amines formation in cooked beef patties. *Journal of Food Science,* 76(8), T174-
603 T180. https://doi.org/10.1111/j.1750-3841.2011.02338.x

604 Quelhas, I., Petisca, C., Viegas, O., Melo, A., Pinho, O., & Ferreira, I.M.P.L.V.O. (2010). Effect of
605 green tea marinades on the formation of heterocyclic aromatic amines and sensory quality of
606 pan-fried beef. *Food Chemistry,* 122(1), 98-104.
607 https://doi.org/10.1016/j.foodchem.2010.02.022.

608 Račkauskienė, I., Pukalskas, A., Venskutonis, P. R., Fiore, A., Troise, A. D., & Fogliano, V. (2015).
609 Effects of beetroot (*Beta vulgaris*) preparations on the Maillard reaction products in milk and
610 meat-protein model systems. *Food Research International,* 70, 31-39.
611 https://doi.org/10.1016/j.foodres.2015.01.026

612 Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant
613 activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology*
614 *and Medicine,* 26(9), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

615 Salazar, R., Arámbula-Villa, G., Hidalgo, F. J., & Zamora, R. (2014). Structural characteristics that
616 determine the inhibitory role of phenolic compounds on 2-amino-1-methyl-6-
617 phenylimidazo[4,5-b]pyridine (PhIP) formation. *Food Chemistry,* 151, 480-486.
618 https://doi.org/10.1016/j.foodchem.2013.11.105

619 Sandu, O., Song, K., Cai, W., Zheng, F., Uribarri, J., & Vlassara, H. (2005). Insulin Resistance and
620 Type 2 Diabetes in High-Fat–Fed Mice Are Linked to High Glycotoxin Intake. *Diabetes,* 54(8)
621 2314-2319; https://doi.org/10.2337/diabetes.54.8.2314

622 Shirai, T., Sano, M., Tamano, S., Takahashi, S., Hirose, M., Futakuchi, M., . . . Ito, N. (1997). The
623 prostate: A target for carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
624 (PhIP) derived from cooked foods. *Cancer Research,* 57(2), 195-198.

625 Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and
626 other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in*
627 *Enzymology,* 299, 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

628 Skog, K. I., Johansson, M. A. E., & Jägerstad, M. I. (1998). Carcinogenic heterocyclic amines in
629 model systems and cooked foods: A review on formation, occurrence and intake. *Food and*
630 *Chemical Toxicology,* 36(9), 879-896. https://doi.org/10.1016/S0278-6915(98)00061-1

631 Totlani, V. M., & Peterson, D. G. (2006). Epicatechin carbonyl-trapping reactions in aqueous
632 Maillard systems: Identification and structural elucidation. *Journal of Agricultural and Food*
633 *Chemistry,* 54(19), 7311-7318. https://doi.org/10.1021/jf061244r

634 Totlani, V. M., & Peterson, D. G. (2005). Reactivity of epicatechin in aqueous glycine and glucose
635 Maillard reaction models: Quenching of C2, C3, and C4 sugar fragments. *Journal of Agricultural*
636 *and Food Chemistry,* 53(10), 4130-4135. https://doi.org/10.1021/jf050044x

637 Uribarri, J., Cai, W., Peppa, M., Goodman, S., Ferrucci, L., Striker, G., & Vlassara, H. (2007).
638 Circulating glycotoxins and dietary advanced glycation endproducts: two links to inflammatory
639 response, oxidative stress, and aging. *The journals of gerontology. Series A, Biological sciences*
640 *and medical sciences*, 62(4), 427-433. https://www.ncbi.nlm.nih.gov/pubmed/17452738

641 Wong, D., Cheng, K. W., & Wang, M. (2012). Inhibition of heterocyclic amine formation by water-
642 soluble vitamins in Maillard reaction model systems and beef patties. *Food Chemistry,* 133(3),
643 760-766. https://doi.org/10.1016/j.foodchem.2012.01.089

644 Yoshimura, T., Harashima, M., Kurogi, K., Suiko, M., Liu, M., & Sakakibara, Y. (2016). A novel
645 procedure for the assessment of the antioxidant capacity of food components. *Analytical*
646 *Biochemistry,* 507, 7-12. https://doi.org/10.1016/j.ab.2016.05.002

647 Yu, C., Shao, Z., Zhang, J., Liu, B., Kong, L., Zhang, Y., & Wang, S. (2018). Dual effects of creatinine
648 on the formation of 2-amino-1-methyl-6-phenylimidazo 4,5-b]pyridine (PhIP). *Journal of Food*
649 *Science,* 83(2), 294-299. https://doi.org/10.1111/1750-3841.14035

650 **Table 1–The yield,** total phenolic content **and antioxidant activity of different extracts of** lingonberry leaves**.**

LL Extract	Yield (g/100 g pdw)	TPC (mg GAE/edw)	TPC (mg GAE/g pdw)	DPPH, EC_{50} (mg/mL)	ABTS (mM TE/g edw)	ABTS (mM TE/g pdw)	ORAC (mM TE/g edw)	ORAC (mM TE/g pdw)
WE	32.96±1.90^{d}	252.22±4.40^{c}	83.12±1.45^{d}	2.40±0.01^{a}	11.16±0.55^{d}	3.68±0.18^{d}	10.63±0.88^{e}	3.50±0.29^{e}
ME	36.38±2.51^{e}	250.56±3.62^{c}	91.15±1.31^{e}	2.36±0.00^{a}	6.05±1.55^{c}	2.20±0.57^{c}	7.44±1.05^{d}	2.71±0.38^{d}
AE	23.69±2.60^{c}	242.42±5.05^{c}	57.42±1.19^{c}	2.52±0.01^{a}	4.33±0.23^{b}	1.03±0.05^{b}	4.43±0.23^{b}	1.44±0.10^{c}
DME	14.09±0.60^{b}	141.80±3.91^{b}	19.98±0.55^{b}	2.54±0.00^{a}	3.07±0.63^{b}	0.43±0.09^{a}	6.08±0.42^{c}	0.62±0.03^{b}
DAE	9.57±0.50^{a}	96.52±8.53^{a}	9.24±0.82^{a}	5.11±0.58^{b}	1.32±0.53^{a}	0.13±0.05^{a}	1.62±0.09^{a}	0.16±0.00^{a}

651 Data represent mean ± SD of triplicate analyses: different superscript letters ($^{a–e}$) with in a column indicate significant differences
652 between the extracts ($P < 0.05$). Abbreviations: LL, lingonberry leaves; TPC, total phenolic content; WE, water extract; ME, methanol
653 extract; AE, acetone extract; DME, deodorized methanol extract; DAE, deodorized acetone extract; edw, extract dry weight; pdw,
654 plant dry weight.

655

656 **Table 2– Compounds identified in the extracts of *Vaccinium vitis-idaea* L. leaves and their UPLC/qTOF–MS/MS data and the**

657 **content**

No.	Compound	t_R (min)	$[M-H]^-$ (*m/z*)	Molecular ion formula	MS/MS (*m/z*)	Lingonberry leaves extracts				
						WE	ME	AE	DME	DAE
1	Catechin[1]	1.28	289.0719	$C_{15}H_{13}O_6$	245.0826(100), 179.0306(25)	31.85±0.23^c	32.18±0.56^c	36.48±0.31^d	25.37±0.08^b	5.93±0.15^a
2	Epicatechin[2]	1.55	289.0716	$C_{15}H_{13}O_6$	245.0825(60), 179.0306(19)	14.62±0.35^a	32.18±0.57^b	14.33±0.16^a	18.56±9.5^{ab}	1.53±0.01^a
	Total Flavan-3-ols					***46.47***	***64.36***	***50.81***	***43.93***	***7.46***
3	Quinic acid[1]	0.35	191.0564	$C_7H_{11}O_6$	–	42.81±2.52^c	25.46±0.56^b	2.00±0.02^a	1.61±0.12^a	TR
4	Citric acid[1]	0.43	191.0201	$C_6H_7O_7$	111.0083(20)	46.48±0.32	ND	ND	ND	ND
5	Caffeoyl-shikimic acid[2]	1.08	335.0775	$C_{20}H_{15}O_5$	179.0340(55), 161.0237(100)	2.85±0.02^e	2.22±0.10^d	1.96±0.02^c	1.25±0.02^b	0.72±0.05^a
6	Chlorogenic acid[1]	1.33	353.0874	$C_{16}H_{17}O_9$	–	3.44±0.05^a	TR	TR	TR	ND
7	Caffeic acid[1]	1.47	179.0353	$C_9H_7O_4$	–	2.62±0.04^b	ND	ND	1.04±0.00^a	0.98±0.08^a
8	*p*-Coumaric acid[1]	1.87	163.0402	$C_9H_7O_3$	–	1.56±0.16^b	ND	ND	0.92±0.02^a	1.57±0.04^b
	Total hydroxycinnamic acids					***99.76***	***27.68***	***3.96***	***4.82***	***3.27***
9	Arbutin[2]	0.49	271.0824	$C_{12}H_{15}O_7$	108.0218(100), 109.0275(8)	33.18±0.03^d	31.78±0.67^d	23.00±0.43^c	17.48±0.08^b	9.61±0.05^a
10	2-*O*-caffeoylarbutin[2,3]	1.70	433.1147	$C_{21}H_{21}O_{10}$	323.0778 (5), 179.0350 (30), 161.0245 (100), 135.0449 (10)	24.11±1.01^c	29.85±0.35^d	34.34±0.24^e	21.92±0.07^b	10.21±0.23^a
11	Caffeoyl acetyl arbutin[3]	2.56	475.1242	$C_{23}H_{23}O_{11}$	179.0347(15), 161.0244(100)	8.51±0.14^b	8.82±0.00^b	10.97±0.22^c	10.49±0.31^c	6.05±0.08^a
	Total Arbutin derivatives					***65.8***	***70.45***	***68.31***	***49.89***	***25.87***
12	B-type proanthocyanidin[2]	1.34	577.1336	$C_{30}H_{25}O_{12}$	407.0757(68), 289.0709(100), 245.0807(21), 161.0233(15), 125.0240(35)	8.35±0.35^a	TR	TR	TR	ND
13	A-type proanthocyanidin[2,3]	1.95	575.1195	$C_{30}H_{23}O_{12}$	539.0998(65), 407.0791(48), 285.0387(100), 125.0245(58)	3.67±0.03^c	0.66±0.03^b	0.66±0.19^b	0.49±0.00^{ab}	0.27±0.07^a
	Total proanthocyanidins					***12.02***	***0.66***	***0.66***	***0.49***	***0.27***
14	Rutin[1]	1.84	609.1459	$C_{27}H_{29}O_{16}$	300.0267(100), 301.0332(30)	1.53±0.03^a	TR	TR	TR	ND
15	Quercetin-3-*O*-glucoside[1]	1.95	463.0881	$C_{21}H_{19}O_{12}$	301.0339(66), 300.0272(100)	3.33±0.05^b	3.31±0.05^b	3.15±0.20^b	3.26±0.11^b	1.12±0.09^a

16	Quercetin pentoside 1[2,3]	2.10	433.0773	$C_{20}H_{17}O_{11}$	301.0337(56), 300.0266(100), 271.0225(5)	20.43±0.07^{c}	ND	ND	18.20±0.32^{b}	7.70±0.25^{a}
17	Quercetin pentoside 2[2,3]	2.25	433.0769	$C_{20}H_{17}O_{11}$	301.0345(58), 300.0274(100), 271.0238(5)	7.71±0.28^{b}	7.44±0.11^{b}	7.68±0.16^{b}	9.34±0.26^{c}	4.17±0.17^{a}
18	Quercetin-deoxyhexoside[2,3]	2.28	447.0931	$C_{21}H_{19}O_{11}$	301.0338(23), 300.0252(100), 271.0227(86)	15.95±0.08^{e}	15.24±0.18^{d}	12.38±0.14^{c}	10.80±0.07^{b}	7.70±0.00^{a}
19	Quercetin-3-*O*-(HMG)-rhamnoside[3]	2.67	591.1355	$C_{27}H_{27}O_{15}$	529.1334(27), 489.1028(100), 447.0924(72), 301.0269(65)	29.80±0.10^{d}	0.41±0.03^{b}	0.61±0.02^{c}	0.29±0.01^{a}	0.30±0.01^{a}
20	Kaempferol-3-*O*-(HMG)-rhamnoside[3]	2.95	575.1392	$C_{27}H_{27}O_{14}$	515,1377(15), 473.1075(40), 431.0969(38), 285.0391(100)	1.78±0.02^{d}	1.07±0.02^{b}	1.46±0.08^{c}	1.02±0.06^{b}	0.38±0.01^{a}
	Total flavonols					***80.53***	***27.47***	***25.28***	***42.91***	***21.37***
	Total phenols					**304.58**	**190.62**	**149.02**	**142.04**	**58.24**

Data represent mean (AU×10^{-7}/mg edw) ± SD of triplicate analyses: different superscript letters ($^{a–e}$) with in a row indicate significant differences between the extracts ($P < 0.05$). [1]Compounds identified by comparing with standard. [2]Compounds identified tentatively by calculated molecular formula and fragmentation patterns using METLIN and/or HMDB databases. [3]Compounds identified tentatively by calculated molecular formula and fragmentation patterns comparing to literature data (Ek et al., 2006; Liu et al., 2017). Abbreviations: WE, water extract; ME, methanol extract; AE, acetone extract; DME, deodorized methanol extract; DAE, deodorized acetone extract; edw, extract dry weight; NI, not identified; –, not performed; ND, not detected; TR, traces.

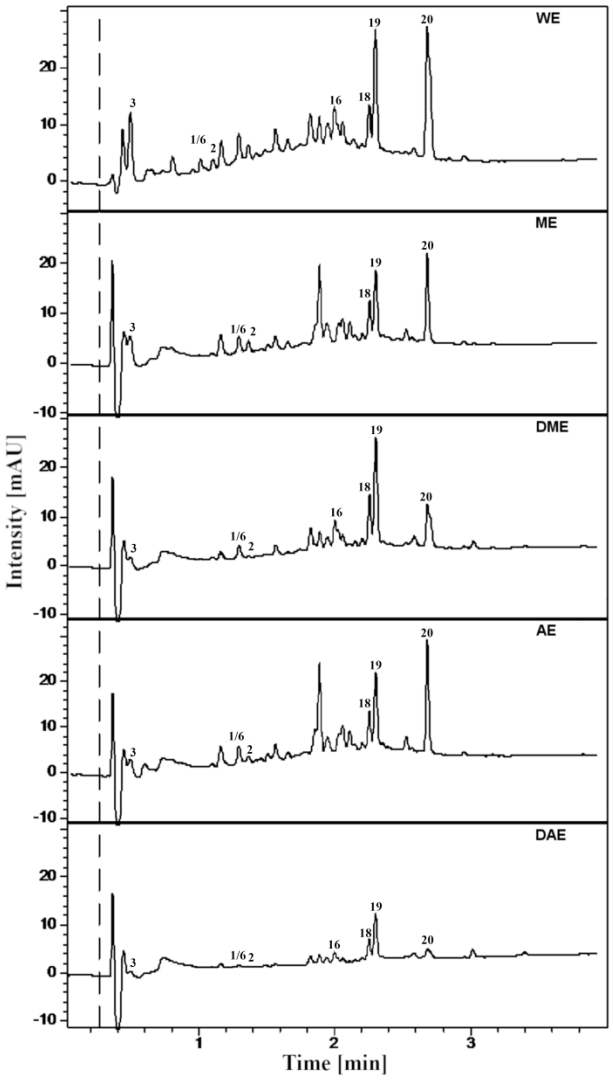
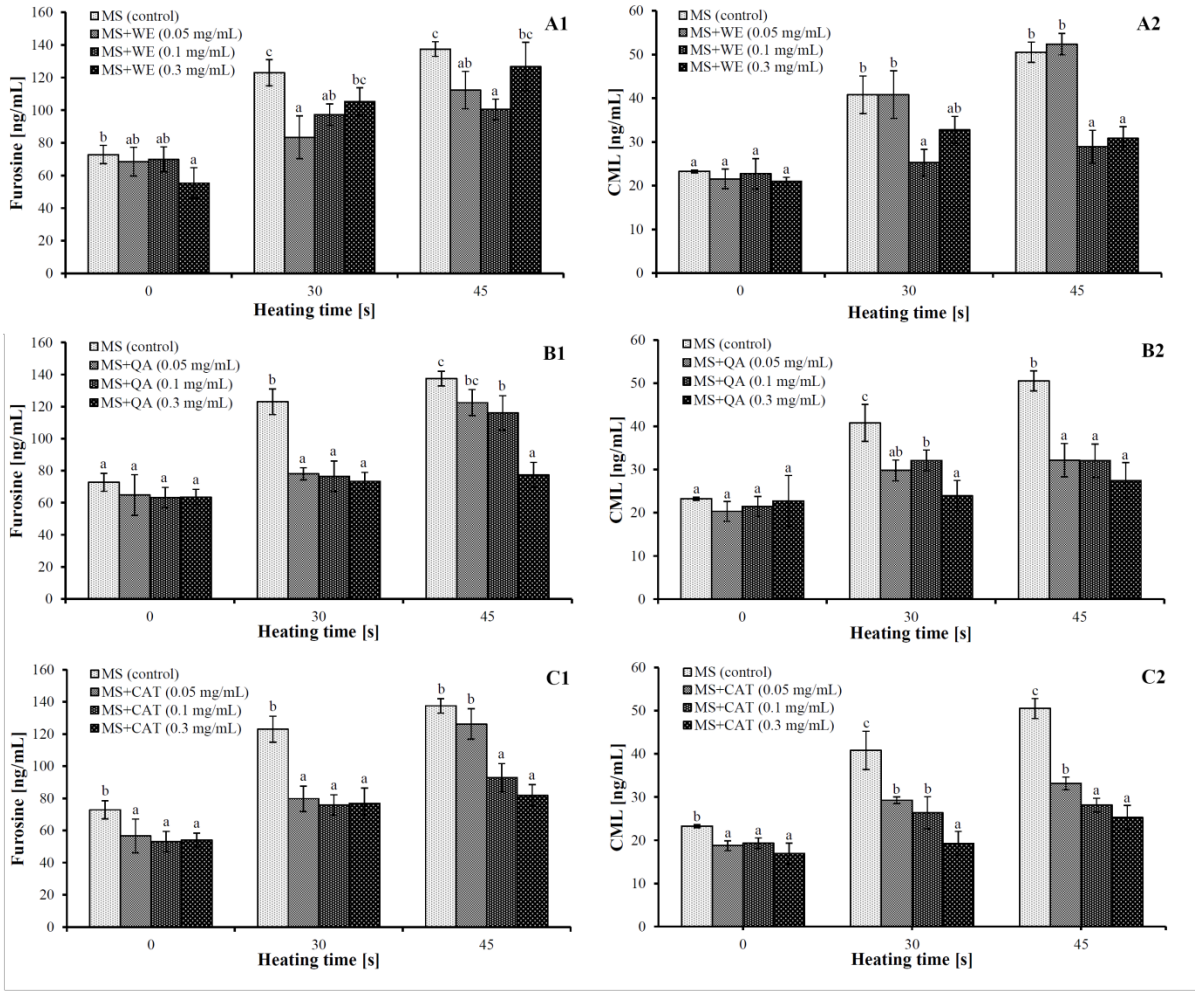


Figure 1–UPLC-UV chromatograms at 254 nm of lingonberry leaf extracts. Peak numbers correspond to the compounds listed in Table 2. Abbreviations: WE, water extract; ME, methanol extract; AE, acetone extract; DME, deodorized methanol extract; DAE, deodorized acetone extract.



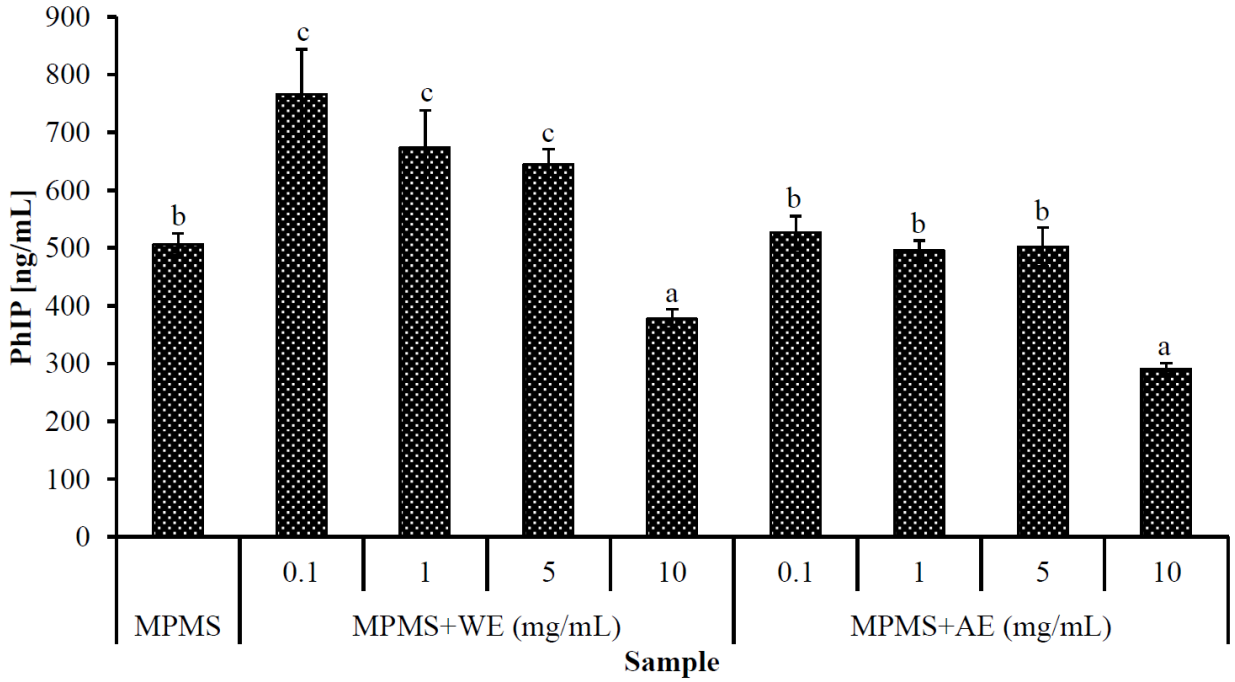
672

673 **Figure 2**–Effects of WE, quinic acid and catechin on furosine and CML formation in milk model

674 system at different heating times: the different letters on the columns at the same heating time

675 indicate the significant differences ($P < 0.05$). Abbreviations: MS, milk model system; QA, quinic

676 acid; CAT, catechin; WE, water extract.



677

678 **Figure 3**–Effects of WE and AE of lingonberry leaves on PhIP formation in meat-protein model

679 system. The different letters on the columns indicate the significant differences ($P < 0.05$).

680 MPMS: meat-protein model system; WE: water extract; AE: acetone extract.

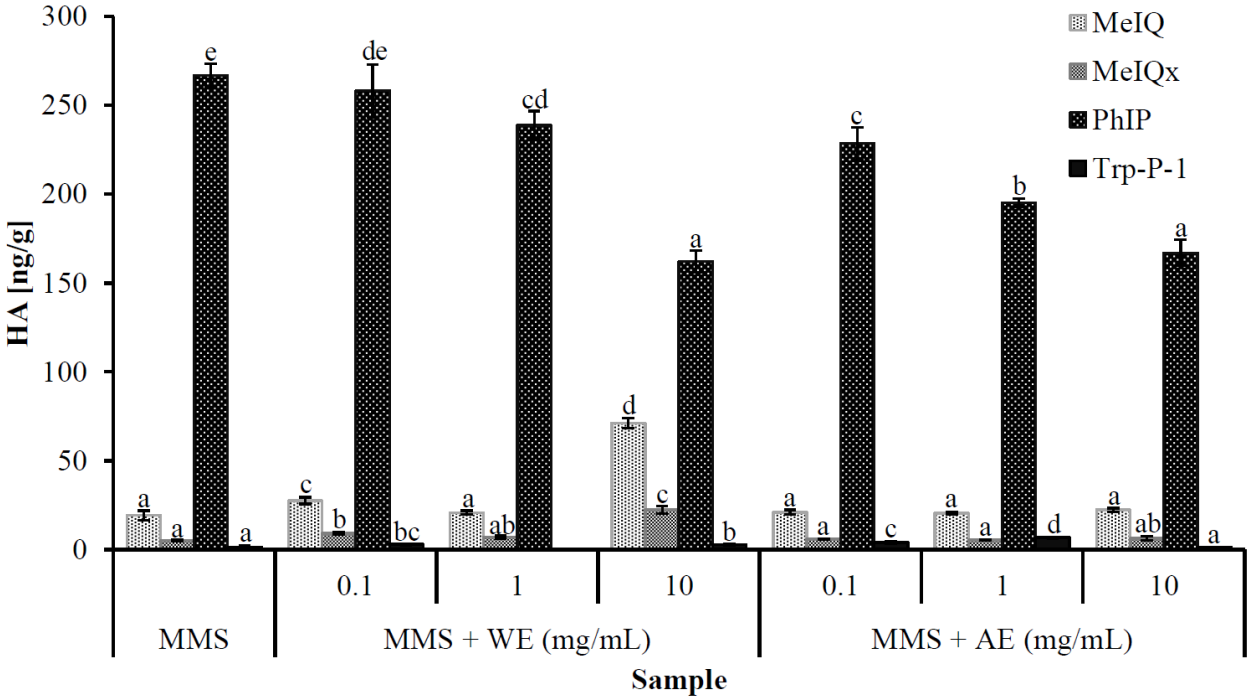


Figure 4–Effects of WE and AE of lingonberry leaves on HAs formation in meat model system. The different letters on the columns at the same HA indicate the significant differences ($P < 0.05$). MMS: meat model system; WE: water extract; AE: acetone extract.